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Structure and Dynamics of the Actin Filament

Jim Pfaendtner[1,2], Edward Lyman[1,2], Thomas D. Pollard[3,4,5] and Gregory A. Voth[1,2]*

[1]*Center for Biophysical Modeling and Simulation, University of Utah, 315 South 1400 East, Salt Lake City, UT 84112-0850, USA*

[2]*Department of Chemistry, University of Utah, 315 South 1400 East, Salt Lake City, UT 84112-0850, USA*

[3]*Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06520-8103, USA*

[4]*Department of Cell Biology, Yale University, New Haven, CT 06520-8103, USA*

[5]*Department Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520-8103, USA*

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We used all-atom molecular dynamics simulations to investigate the structure and properties of the actin filament, starting with either the recent Oda model or the older Holmes model. Simulations of monomeric and polymerized actin show that polymerization changes the nucleotide-binding cleft, bringing together the Q137 side chain and bound ATP in a way that may enhance the ATP hydrolysis rate in the filament. Simulations with different bound nucleotides and conformations of the DNase I binding loop show that the persistence length of the filament depends only on loop conformation. Computational modeling reveals how bound phalloidin stiffens actin filaments and inhibits the release of γ-phosphate from ADP-P_i actin.

Keywords: cytoskeleton; ATP hydrolysis; persistence length; filament; actin

Introduction

Actin is a highly conversed protein that is abundant in eukaryotic cells and known to play a role in a wide range of cellular functions. Actin is chiefly found in two forms: monomeric (or globular) actin and filamentous actin (the polymerized counterpart). The dynamic equilibrium between the two forms is controlled by a variety of factors within the cell.[1] Actin filaments are a central structural feature of all muscle tissue,[2] and controlled polymerization of branched networks of actin filaments produces force for cell motility.[3]

The structural and dynamic properties of actin monomers and filaments depend, at least in part, on the state of the bound nucleotide.[4–7] Both forms of actin strongly bind ATP or ADP, but polymerized actin hydrolyzes its bound ATP about 40,000-fold faster[8] than monomeric actin,[9] but the mechanism is a major unsolved challenge.[10] It is assumed that

**Corresponding author.* Center for Biophysical Modeling and Simulation, University of Utah, 315 South 1400 East, Salt Lake City, UT 84112-0850, USA. E-mail address: voth@hec.utah.edu.

Abbreviations used: MD, molecular dynamics; Arp, actin-related protein; DB loop, DNase I binding loop; P_i, inorganic phosphate; CG, coarse-grained; PDB, Protein Data Bank.

changes in the nucleotide-binding cleft increase the ATPase activity of polymerized actin. Previous molecular dynamics (MD) studies of actin and actin-related proteins (Arps) showed that the conformation of the nucleotide-binding cleft depends on the bound nucleotide in monomeric actin and Arps[11–13] and in actin trimers.[12] However, these findings do not address the means by which polymerization changes the rate of ATP hydrolysis. Experimental data[14,15] showed that residue Q137 is important in ATP hydrolysis, and Oda *et al.* hypothesized that flattening of the actin molecule moves residue Q137 closer to the bound ATP.[16] Crystal structures of monomeric actin[5,6] show that Q137 coordinates a water molecule that might attack the bound ATP.

Several dozen crystal structures of actin monomers have been determined since the first crystal structure of ATP-actin bound to DNase I;[17] however, currently, no high-resolution crystal structures of filamentous actin are available. The parameters of the actin helix were originally established by X-ray diffraction of whole muscle[18,19] and by electron microscopy.[20–22] The first model of filamentous actin that was built with a crystal structure of the subunit is now commonly known as the Holmes model.[23] Recently, Oda *et al.* obtained higher-resolution fiber diffraction data from filamentous actin stabilized by phalloidin and aligned with a superconducting magnet.[16] Their refined model has the same helical arrangement as actin subunits, but the actin subunit itself is flatter in the filament than in any crystal structure of monomeric actin. In the Oda filament model, the two halves of the protein (cf., Fig. 1) are rotated by about 20°, so that all four subdomains are close to lying in a plane. Oda *et al.* and Volkmann *et al.*[24] have observed this flattening of the actin subunit in three-dimensional reconstructions of filaments from electron micrographs. These models suggest that flattening of the actin subunit is a main conformational change associated with actin polymerization, and that this change underlies the higher ATPase activity of filamentous actin.[16,25]

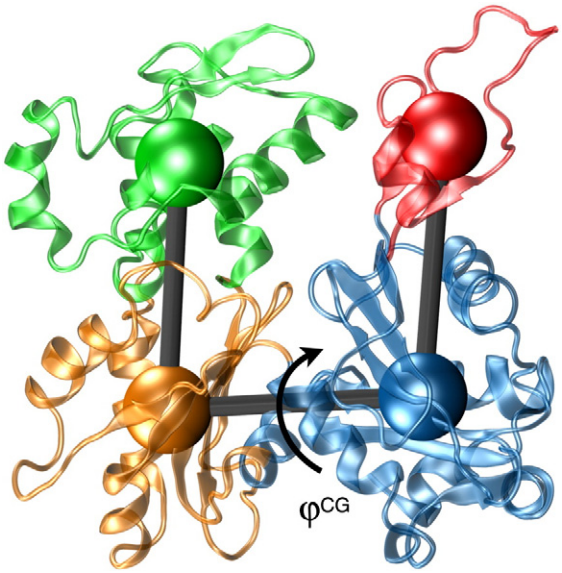

Fig. 1. Actin subunit from the Oda[16] filament model in atomistic (cartoon) and CG representations. The four subdomains shown are as follows: S1 (blue), residues 1–32, 70–144, and 338–375; S2 (red), residues 33–69; S3 (orange), residues 145–180 and 270–337; and S4 (green), residues 181–269. The cartoon representation of the protein is obtained from PDB entry 2ZWH, and the "flatness" order parameter used for analysis is labeled.

The conformation of the DNase I binding loop (DB loop) in actin filaments is unsettled. The observation that the DB loop of some[6]—but not all[9]—crystals of ADP-bound actin forms a well-defined α-helix led to the idea that DB loop folds into a helix in conjunction with ATP hydrolysis and phosphate release. The Oda model is based on a form of monomeric actin with an unstructured DB loop,[5,17] but Oda *et al.* proposed that an extension of the helical DB loop into the neighboring actin subunit in filaments could also explain the observed diffraction patterns.[16] This is an intriguing possibility given the evidence that release of inorganic phosphate (P_i) subsequent to ATP hydrolysis within filamentous actin softens the filament[4,26] and increases susceptibility to attack by severing proteins.[27] Additionally, computational studies[26] have drawn comparisons between the ATP-bound form (DB loop unstructured) and the ADP-bound form (DB loop folded) of filamentous actin. A recent computational study of monomeric and trimeric actins demonstrated that folding of the DB loop in ADP-actin is thermodynamically favorable.[12]

Motivated by unanswered questions concerning the structure and properties of actin filaments, we present a large-scale all-atom MD study of the Oda and Holmes filament models with variations in both the bound nucleotide and the conformation of the DB loop. Previous MD simulations of actin and Arps[11–13,26,28,29] explored the structure and dynamics of actin on timescales ranging from a few nanoseconds to 100 ns. Actin and Arp simulations based on purely classical MD methods investigated the short timescale properties of the actin monomer or filament, especially the conformation of the DB loop and the properties of the nucleotide-binding cleft. Although MD simulations are limited to short timescales, they offer a complementary perspective to experimental techniques, inasmuch as they are fully atomistic in character and therefore permit sampling and observation of all atomic degrees of freedom in the system.

We performed MD simulations in an explicitly solvated aqueous environment of nine different actin filaments based on either the Holmes model or the Oda model with bound ATP or ADP and either folded or unfolded DB loops, including an additional simulation with bound phalloidin to further replicate the experimental conditions under which the filament models were obtained. We investigated the equilibrium dynamics of each of these filaments with an emphasis on understanding how polymerization stimulates the ATP hydrolysis rate and how the bound nucleotide influences the